

Table 1

## Cells

Cell	Receptor
<b>5.25</b>	<b>CXCR4, CD4, CCR5 (not expressed well) BONZO</b>
<b>5.25.Luc4.M7</b>	<b>CD4, CCR5, BONZO</b>
<b>HOS.CD4.CCR5</b>	<b>CD4, CCR5</b>
<b>HOS.CD4.CXCR4</b>	<b>CD4, CXCR4</b>
<b>HOS.CD4</b>	<b>CD4, low level expression of CCR5 and CXCR4</b>
<b>HOS HT4 R5 GFP wt</b>	<b>CD4, CXCR4, CCR5</b>
<b>HOS.CD4.CCR5.GFP.M7#6*</b>	<b>CD4, CXCR4, CCR5</b>
<b>P4.CCR5</b>	<b>CD4, CXCR4, CCR5</b>
<b>U87.CD4</b>	<b>CD4</b>
<b>U87.CD4 R5</b>	<b>CD4, CCR5</b>
<b>U87.CD4 X4</b>	<b>CD4, CXCR4</b>
<b>MT2</b>	<b>CD4, CXCR4</b>
<b>MT4</b>	<b>CD4, CXCR4</b>
<b>PM1</b>	<b>CD4, CXCR4, CCR5</b>
<b>CEM NKr CCR5</b>	<b>CD4, CXCR4, CCR5</b>

**Table 2** Representative viruses and reagents

Viruses	Envelope <sup>a</sup>	Source
89.6, SF2	R5-X4/SI/B	ARRRP <sup>b</sup>
92BR014, 92US076	R5-X4/SI/B	ARRRP
JR-CSF, 91US005	R5/NSI/B	ARRRP
91US054	SI/B	ARRRP
NL43, MN, ELI	X4/B	ARRRP
92HT599	X4	ARRRP
92UG031	R5/NSI/A	ARRRP (IN-HOUSE)
92TH014, 92TH026	R5/NSI/B	ARRRP (IN-HOUSE)
92BR025, 93MW959	R5/SI/C	ARRRP (IN-HOUSE)
92UG035	R5/NSI/D	ARRRP (IN-HOUSE)
92TH022, 92TH023	R5/NSI/E	ARRRP (IN-HOUSE)
93BR020	R5-X4/SI/F	ARRRP (IN-HOUSE)
Antibodies	Epitope <sup>c</sup>	Source
Mabs 2F5, 1577	gp41 TM	ARRRP
Mabs IG1b12, 2G12, 17b, 48D	gp120 SU	ARRRP
Neutralization sera #2, HIV-IG	Polyclonal	ARRRP
Entry inhibitors	Target	Source
CD4-TG	gp120 SU	Genentech
CD4-IGG2	gp120 SU	Adarc
SCD4	Sigma	Progenics
T20 (DP178)	gp41 TM	Trimeris
Rantes, MIP1a/b	CCR5	SIGMA/ARRRP
SDF1a/b	CXCR4	SIGMA/ARRRP
AMD 3100	CXCR4	AnorMed
Dextran sulfate, Heparin	Non-specific	Sigma

<sup>a</sup>R5 (CCR5 co-receptor), X4 (CXCR4 co-receptor)

SI (syncytium inducing), NSI (non-syncytium inducing), A,B,C,D,E,F  
(envelope clade designation)

<sup>b</sup>AIDS Research and Reference Reagent Program

## Table 3

Primers Tested for the Amplification of HIV Envelope	
<b>RT PRIMERS</b>	
RT env_N3	5'-GGA GCA TTT ACA AGC AGC AAC ACA GC-3'
RT env 9720	5'-TTC CAG TCA VAC CTC AGG TAC-3'
RT env 9740	5'-AGA CCA ATG ACT TAY AAG G-3'
<b>5' PCR PRIMERS</b>	
5'env	5'-GGG CTC GAG ACC GGT CAG TGG CAA TCA GAG TGA AG- 3'
5'env_Xho/Pin	5'-GGG CTC GAG ACC GGT GAG CAG AA-3 ACA GTG GCA ATG A-3'
5'env_START	5'-GGG CTC GAG ACC GGT GAG CAG AAG ACA GTG GCA ATG -3'
<b>3' PCR PRIMERS</b>	
3' env	5' -GGG TCT AGA ACG CGT TGC CAC CCA TCT TAT AGC AA- 3'
3'env_Xba/Mlu	5'-GGG TCT AGA ACG CGT CCA CTT GCC ACC CAT BTT ATA GC-3'
3'env_STOP	5'-GGG TCT AGA ACG CGT CCA CTT GCC ACC CAT BTT A-3'
3' delta CT	5' -GAT GGT CTA AGA CGC TGT TCA ATA TCC CTG CCT AAC TC- 3'
All Experiments are located in Virologic Book number 0188	

# Table 4 (Panel 1)

Anti-HIV Drugs			
Drug/Compound	Generic Name	Trademark	Manufacturer
<b>RT Inhibitors (NRTI, nucleotide analogs)</b>			
AZT, ZDV	Zidovudine	Retrovir	Glaxo/Wellcome
3TC	Lamivudine	Epivir	Glaxo/Wellcome
AZT + 3TC		Combivir	Glaxo/Wellcome
d4T	Stavudine	Zerit	Bristol-Myers/Squibb
ddl	Didanosine	Videx	Bristol-Myers/Squibb
ddC	Zalcitabine	Hivid	Hoffman La Roche
1592U89	Abacavir	Ziagen	Glaxo/Wellcome
AZT + 3TC + 1592U89		Trizivir	Glaxo/Wellcome
(-)FTC (5-fluoro-3TC; Corviracil)	Emtricitabine		Triangle Pharmaceuticals
(-)FTC + (+)FTC (50:50)	Racimir		QuadPharma
DAPD (DXG active)	Amdoxovir		Triangle Pharmaceuticals
F-ddA (2-fluoro-ddA)	Lodenosine		MedImmune Oncology (US Bioscience)
BCH-10652, dOTC (2-deoxy-3-oxa-4-thiocytidine)			BioChem Pharma, Inc.
D-d4FC			Triangle Pharmaceuticals (Schinazi)
<b>RT Inhibitors (NTRT, nucleotide analogs)</b>			
bis-POC PMPA (GS-4331)	Tenofovir		Gilead Sciences
bis-POM PMEA (GS-840)	Adefovir dipivoxil		Gilead Sciences
<b>RT Inhibitors (NNRTI, non-nucleosides)</b>			
BI-RG-587	Nevirapine	Viramune	Boehringer/Ingleheim (Roxanne)
BHAP PNU-90152T	Delavirdine	Rescriptor	Pharmacia & Upjohn
DMP 266 (L-743,726)	Efavirenz	Sustiva	Dupont Pharmaceuticals (Avid)
MKC442 (Coactinon)	Emivirine		Triangle/Mitsubishi Kasei
AG-1549 (S1153) (on hold)	Capravirine		Agouron Pharmaceuticals
PNU-142721			Pharmacia & Upjohn
DPC-961, -963, -083, -08?			DuPont Pharmaceuticals
SJ-3366	Also entry inhibitor?		Samjin Pharmaceuticals
BHAP PNU-87201	Ateviridine		Upjohn
GW420867X (quinoxaline)	(2 <sup>nd</sup> gen. HBY 097)		Glaxo/Wellcome (Hoechst Bayer)
TMC 120 (R147681)			Tibotec
TMC 125 (R165335)			Tibotec
R86183	tivirapine		Janssen Pharmaceuticals
Calanolide A			Sarawak Medichem Pharmaceuticals
<b>Protease Inhibitors (PRI)</b>			
Ro 31-8959	Saquinavir-(hgc) Saquinavir-(sgc)	Invirase Fortivase	Hoffman-La Roche
MK-639 (L-735,524)	Indinavir	Crixivan	Merck Research Laboratories
ABT-538 (A-84538)	Ritonavir	Norvir	Abbott Laboratories
AG1343	Nelfinavir	Viracept	Agouron Pharmaceuticals
141W94 (VX-478)	Amprenavir	Agenerase	Glaxo-Wellcome/Vertex
ABT-378/r	Lopinavir/ritonavir	Kaletra	Abbott Laboratories
BMS 232,632 (aza-peptide)			Bristol-Myers-Squibb
PNU-140690	Tipranavir		Pharmacia & Upjohn
DMP 450 (cyclic urea)	Mozenavir		Triangle/Avid (ph I/II)
TMC 126 (Erickson's compound)			Tibotec
G/W433908 (VX-175)	amprenavir pro-drug		Glaxo/Wellcome/Vertex
L756,423 (on hold)			Merck
PD-178390 (dihydropyrone)			Parke Davis (Boehringer-Ingleheim)
? new candidate			Roche
DPC 681 and 684			DuPont Pharmaceuticals
AG-1776 (JE-2147 = KNI-764)			Agouron Pharmaceuticals
<b>Envelope/Receptor Inhibitors</b>			
T-20 (gp41)	Pentafuside		Trimeris Pharmaceuticals
T-1249 (gp41)			Trimeris Pharmaceuticals
D-peptide inhibitor (gp41) small mol.	SCH-C		Schering-Plough
AMD-3100 (CXCR4)	(bicyclam)		AnorMED
AMD-8664 (CXCR4)	(macrocyclam)		AnorMED
ALX40-4C (CXCR4)			U. PA
FP21399			Fuji Pharmaceuticals
PRO 542 (gp120)	CD4IgG2		Progenics Pharmaceuticals
PRO-140 (CCR5)	MAb CCR5		Progenics Pharmaceuticals
T-22 (CXCR4)	(peptide, 18-mer)		
Met-SDF-1 (CXCR4)			
TAK 779 (CCR5 antagonist)			Takeda
AOP-Rantes (CCR5)			Gryphon Sciences

# Table 4 (Panel 2)

Rantes 9-68 (CCR5)			
CCR5 antagonists	4-(piperidin-1-yl) butane class		Merck
$\alpha$ -Immunokine-NNS03 (CCR5, CXCR4)	$\alpha$ -cobratoxin		PhyloMed Corp.
<b>Integrase Inhibitors</b>			
AR-177	Zintevir		Aronex Pharmaceuticals
Diketo acids			Merck Research Laboratories
<b>Nucleocapsid Inhibitors</b>			
RB 2121	cyclic peptide p7 mimic		(see PNAS 96:4886-4891 (1999))
CI-1012			Achelion Pharmaceuticals
<b>RNase H Inhibitor</b>			
SP1093V (BBNH Fe+3 derivative)			(Parniak)

FDA approved drugs are shown in **boldface**, red = discontinued development, blue = not sure about development status

**Table 4 (Panel 3)**

<b>Generic Name (abbreviation)</b>	<b>Brand Name</b>	<b>Firm</b>	<b>FDA Approval Date</b>
zidovudine, AZT	Retrovir	Glaxo Wellcome	March 87
didanosine, ddI	Videx	Bristol Myers-Squibb	October 91
zalcitabine, ddC	Hivid	Hoffman-La Roche	June 92
stavudine, d4T	Zerit	Bristol Myers-Squibb	June 94
lamivudine, 3TC	Epivir	Glaxo Wellcome	November 95
saquinavir, SQV, hcg	Invirase	Hoffman-La Roche	December 95
saquinavir, SQV, sgc	Fortovase	Hoffman-La Roche	November 97
ritonavir, RTV	Norvir	Abbott Laboratories	March 96
indinavir, IDV	Crixivan	Merck & Co., Inc.	March 96
nevirapine, NVP	Viramune	Boehringer Ingelheim	June 96
nelfinavir, NFV	Viracept	Agouron Pharmaceuticals	March 97
delavirdine, DLV	criptor	Pharmacia & Upjohn	April 97
ZDV+3TC	Combivir	Glaxo Wellcome	September 97
efavirenz, EFV	Sustiva	DuPont Pharmaceuticals	September 98
abacavir, ABC	Ziagen	Glaxo Wellcome	February 99
amprenavir	Agenerase	Glaxo Wellcome	April 99
lopinavir/ritonavir	Kaletra	Abbott	September 2000
ZDV+3TC+ABC	Trizivir	GlaxoSmithKline	November 2000

**CORECEPTOR ASSAY SCREEN TEMPLATE**

active RLU limit: 100 tropism ratio limit: 5 CXCR4 CCR5 DUAL

drug 1: L83

drug 2: AMD

No Drug RLU	1			2			3			4			5			6		
	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4
No Drug RLU	34	26,471	0 00	14,140	55	257 09	46	6,849	0 01	21,656	38,144	0 57	30	7,336	0 00	3,988	31	128 6
L83 RLU		19,258		54				4,696		27	25,542			5,468		34		
AMD RLU		32		12,186				40		7,226	32		39		3,664			
	13			14			15			16			17			18		
No Drug RLU	4,552	19,102	0 24	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4
L83 RLU	28	9,956		149	226	0 66	67,389	72	935 96	611	19,715	0 03	46	3,948	0 01	284	4,330	0 0
AMD RLU	838	34		40	153		99			30	14,985			2,953		38	2,918	
				41	24		42,295			76	1,022			152		25	48	
	25			26			27			28			29			30		
No Drug RLU	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4
L83 RLU	67,828	68	997,47	14,982	12,020	1 25	3,788	4,384	0 86	50	37	██████████	658	242	2 72	231	35	6 6
AMD RLU	129			111	10,839		59	3,397			32	221		493	38	219		
				8,580	3,384		2,049	538										
	37			38			39			40			41			42		
No Drug RLU	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4
L83 RLU	100	39	██████████	3,724	2,310	1 61	450	668	0 67	148	24	6.17	32	22	██████████	60	61	
AMD RLU				38	2,656		37	463			33							
				1,984	272		206	72		139								
	49			50			51			52			53			54		
No Drug RLU	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4
L83 RLU	35	107	0 33	83	4,209	0 02	18,099	8,451	2 14	39,257	38	1033,08	5,413	40	135 33	97,733	29	3370 1
AMD RLU	68			3,502			13,896	6,220		85			35			30		
				88			16,980	8,711		27,832			5,043			48,452		
	61			62			63			64			65			66		
No Drug RLU	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4
L83 RLU	3,769	40	94 23	786	48	16 38	340	21	16 19	5,189	43	120 67	4,318	37	116 70	196	51	3 8
AMD RLU	29			39			31			34			28			23		
				2,773			282			5,356			3,461			184		
	73			74			75			76			77			78		
No Drug RLU	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4
L83 RLU	391	26	15 04	98	38	██████████	4,449	28	158 89	4,357	9,102	0 48	6,090	105	58 00	1,866	3,635	0 5
AMD RLU	50						43			34	7,932		73	77		38	2,092	
				340			3,752			906	36		4,473	51		279	27	
	85			86			87			88			89			90		
No Drug RLU	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4	R5	X4	R5:X4
L83 RLU	510	33	15 45	979	59	16 59	491	33	14 88	300	3,815	0 08	297	3,615	0 08	39	61,594	0 0
AMD RLU	42						49			29	3,661		32	2,946		56,739		
				564			376			268	36		256	30			40	

No Drug RLU L83 RLU AMD RLU	7 X4 R5:X4			8 X4 R5:X4			9 X4 R5:X4			10 X4 R5:X4			11 X4 R5:X4			12 X4 R5:X4			
	R5 30	X4 1,217	R5:X4 0.02	R5 49	X4 1,128	R5:X4 0.04	R5 42,206	X4 70	R5:X4 602.94	R5 45	X4 1,159	R5:X4 0.04	R5 44,589	X4 97	R5:X4 60	R5 32	X4 29,118	R5:X4 22,279	
No Drug RLU L83 RLU AMD RLU		19			20			21			22			23			24		
	R5 100	X4 5,204	R5:X4 0.02	R5 11,299	X4 52	R5:X4 217.29	R5 1,273	X4 20	R5:X4 63.65	R5 7,375	X4 10,856	R5:X4 0.68	R5 4,397	X4 7,130	R5:X4 0.62	R5 16,115	X4 50	R5:X4 322.30	
No Drug RLU L83 RLU AMD RLU		31			32			33			34			35			36		
	R5 53	X4 974	R5:X4 0.05	R5 177	X4 32	R5:X4 5.53	R5 24,739	X4 33	R5:X4 749.67	R5 612	X4 45	R5:X4 13.60	R5 2,997	X4 9,695	R5:X4 0.31	R5 416	X4 31	R5:X4 13.42	
No Drug RLU L83 RLU AMD RLU		43			44			45			46			47			48		
	R5 45	X4 338	R5:X4 0.13	R5 714	X4 45	R5:X4 15.87	R5 11,029	X4 18	R5:X4 612.72	R5 2,908	X4 43	R5:X4 67.63	R5 13,997	X4 52	R5:X4 269.17	R5 24,377	X4 34	R5:X4 716.97	
No Drug RLU L83 RLU AMD RLU		55			56			57			58			59			60		
	R5 9,254	X4 23,846	R5:X4 0.39	R5 5,249	X4 20,393	R5:X4 0.26	R5 470	X4 39	R5:X4 12.05	R5 19,175	X4 126	R5:X4 152.18	R5 513	X4 31	R5:X4 16.55	R5 3,264	X4 28	R5:X4 116.57	
No Drug RLU L83 RLU AMD RLU		67			68			69			70			71			72		
	R5 3,853	X4 77	R5:X4 50.04	R5 411	X4 34	R5:X4 12.09	R5 7,857	X4 41	R5:X4 191.63	R5 25,437	X4 38	R5:X4 669.39	R5 17,443	X4 45	R5:X4 387.62	R5 16,707	X4 38	R5:X4 439.60	
No Drug RLU L83 RLU AMD RLU		79			80			81			82			83			84		
	R5 263	X4 38	R5:X4 6.92	R5 3,890	X4 9,577	R5:X4 0.41	R5 2,089	X4 1,698	R5:X4 1.23	R5 475	X4 43	R5:X4 11.05	R5 8,475	X4 38	R5:X4 223.03	R5 4,107	X4 33	R5:X4 124.45	
No Drug RLU L83 RLU AMD RLU		91			92			93			94			95			96		
	R5 443	X4 13,452	R5:X4 0.03	R5 34	X4 128,238	R5:X4 0.00	R5 22	X4 62	R5:X4 ██████████	R5 45	X4 33	R5:X4 ██████████	R5 43	X4 42	R5:X4 ██████████	R5 46	X4 35	R5:X4 ██████████	

Table 4B

	R5 cells	X4 cells	R5:X4
no drug			
R5 inhibitor			
X4 inhibitor			
%inhib by R5 inhibitor			
%inhib by X4 inhibitor			

	R5	X4	R5:X4
no drug			
R5 inhibitor			
X4 inhibitor			
%inhib by R5 inhibitor			
%inhib by X4 inhibitor			


**DUAL or MIXED**  
**DEAD**

	R5	X4	R5:X4
no drug	14,982	12,020	1
R5 inhibitor	111	10,839	
X4 inhibitor	8,580	3,384	
%inhib by R5 inhibitor	99	10	
%inhib by X4 inhibitor	43	72	

	R5	X4	R5:X4
no drug	43	42	
R5 inhibitor			
X4 inhibitor			
%inhib by R5 inhibitor			
%inhib by X4 inhibitor			

DEAD

Table 4C

Plate Repeat End time Start temp. End temp. BarCode  
1 1 6:39:36 PM 21.6 21.7 N/A

0.5 CPS (CPS)  
18

26648	54	6970	37406	7158	38	1386	930	100	1184	112	30488
19248	262	72	18972	3478	3946	5010	46	14	11004	7474	60
106	9038	4002	32	238	26	976	34	38	48	9736	32
38	2000	528	16	16	36	298	52	14	54	52	38
122	2984	7264	40	38	20	23344	15340	44	156	40	32
36	52	28	40	28	40	62	32	38	22	44	28
22	32	28	9858	68	3802	46	11470	1958	42	42	24
30	64	36	3846	3390	57858	12620	126186	68	34	38	36

Plate Repeat End time Start temp. End temp. BarCode  
2 1 6:41:51 PM 21.5 21.6 N/A

0.5 CPS (CPS)  
34

26294	56	6728	38882	7514	24	1048	1326	40	1134	82	27748
18956	190	72	20458	4418	4714	5398	58	26	10708	6786	40
30	15002	4766	42	246	44	972	30	28	42	9654	30
40	2620	808	32	28	86	378	38	22	32	52	30
92	5434	9638	36	42	38	24348	25446	34	96	22	24
44	44	14	46	46	62	92	36	44	54	46	48
30	44	28	8346	142	3468	30	7684	1438	44	34	42
36	54	30	3784	3840	65330	14284	130290	56	32	46	34

Plate Repeat End time Start temp. End temp. BarCode  
3 1 6:44:06 PM 21.6 21.6 N/A

0.5 CPS (CPS)  
16

18590	18	4306	23902	5386	30	924	894	32	660	48	23382
8698	148	34	14088	2880	3142	3160	40	46	7616	2842	32
30	10252	3542	38	172	34	842	30	32	44	7616	34
28	2396	370	28	26	66	172	32	22	28	36	26
64	3784	4822	32	28	24	6188	5702	34	120	34	24
16	28	62	26	28	30	38	38	38	44	62	34
38	52	40	7020	68	1798	62	7324	1076	50	42	32
32	50	70	3824	3138	53670	13088	104608	32	38	50	32

Plate Repeat End time Start temp. End temp. BarCode  
4 1 6:46:20 PM 21.7 21.8 N/A

0.5 CPS (CPS)  
24

19926	46	5086	27182	5550	26	704	974	32	862	44	21176
11214	158	42	15882	3026	2694	2956	26	20	5294	4906	44
56	11426	3252	82	270	52	814	36	40	64	6906	32
32	2916	556	42	30	64	242	38	24	36	42	40
72	3220	7618	36	38	44	12112	10878	32	246	32	42
32	48	22	30	28	24	136	30	56	34	24	24
32	38	40	8844	86	2386	54	4956	1372	50	46	28
22	34	40	3498	2754	59808	11014	65428	34	40	24	18

Plate Repeat End time Start temp. End temp. BarCode  
5 1 6:48:35 PM 21.9 21.9 N/A

0.5 CPS (CPS)  
38

38	28	40	30	38	234	46	30	46	30	34	22
26	20	32	976	190	58	46	24	28	2298	1290	26
42	3260	420	50	36	34	102	26	34	24	40	24
28	378	88	32	34	32	40	42	54	26	30	48
36	62	11690	38	44	32	42	42	26	36	46	42
46	22	44	38	58	32	38	30	32	36	38	12
22	38	52	40	42	26	34	50	200	36	34	36
36	80	52	40	36	40	52	54	22	40	42	36

Plate Repeat End time Start temp. End temp. BarCode  
6 1 6:50:50 PM 21.8 21.9 N/A

0.5 CPS (CPS)  
30

26	28	40	34	40	50	38	44	34	36	40	40
42	28	32	1068	114	38	22	46	30	2268	1280	40
36	3508	656	48	40	34	88	30	34	56	30	36
28	166	56	28	18	40	18	38	32	34	26	32
32	114	5732	32	42	34	38	34	50	34	38	52
36	22	36	40	30	20	30	26	42	40	26	36
46	24	26	32	60	28	32	40	120	36	20	36
32	58	58	32	24	40	50	36	12	38	40	18

Table 4

Plate Repeat End time Start temp. End temp. BarCode  
1 1 ##### 21.7 21.8 N/A

0.5 CPS (CPS)

32

24	14218	56	21586	28	4034	36	52	42088	40	43332	38
4780	174	71276	730	48	326	28	12022	1288	7198	4314	17856
69452	15306	4008	44	792	180	50	182	25292	718	3572	424
72	3922	546	128	44	66	30	900	11984	3194	16106	23794
30	88	18820	40438	4882	105946	8466	4934	470	20386	548	3376
3420	934	344	6268	5012	170	3546	504	8164	22214	15146	19592
344	82	5344	5330	6710	1880	338	4286	2112	466	7752	4078
580	1018	516	318	302	40	414	32	20	52	48	42

Plate Repeat End time Start temp. End temp. BarCode  
2 1 ##### 21.9 21.9 N/A

0.5 CPS (CPS)

22

44	14062	36	21726	32	3942	24	46	42324	50	45846	26
4324	124	63502	492	44	242	172	10576	1258	7552	4480	14374
66204	14658	3568	56	524	282	56	172	24186	506	2422	408
128	3526	354	168	20	54	60	528	10074	2622	11888	24960
40	78	17378	38076	5944	89520	10042	5564	470	17964	478	3152
4118	638	336	4110	3624	222	4160	318	7550	28660	19740	13822
438	114	3554	3384	5470	1892	188	3494	2066	484	9198	4136
440	940	466	282	292	38	472	36	24	38	38	50

Plate Repeat End time Start temp. End temp. BarCode  
3 1 ##### 21.9 22 N/A

0.5 CPS (CPS)

36

36	38	28	28	38	28	36	42	114	40	60	46
32	34	80	26	16	36	30	18	26	30	48	80
126	106	34	42	30	46	22	26	32	58	8	28
26	46	42	38	34	20	18	34	24	36	100	60
28	146	15756	76	32	26	42	52	36	106	34	24
34	50	20	24	32	18	38	18	36	38	50	58
38	38	28	36	80	50	38	44	34	38	30	28
40	30	38	30	24	22	38	36	34	36	22	26

Plate Repeat End time Start temp. End temp. BarCode  
4 1 ##### 21.9 22 N/A

0.5 CPS (CPS)

16

24	70	30	26	26	40	18	32	66	30	60	34
24	46	118	34	26	40	34	26	26	50	36	56
132	116	84	34	34	48	34	38	30	38	36	28
32	30	32	28	26	40	46	28	12	26	46	58
34	84	12036	94	38	34	44	24	38	86	36	36
24	28	42	44	24	28	40	26	48	32	30	150
62	24	58	32	66	26	28	44	32	22	30	28
44	80	60	28	40	28	34	32	36	18	28	

Plate Repeat End time Start temp. End temp. BarCode  
5 1 ##### 22 22 N/A

0.5 CPS (CPS)

34

38	11132	26	6696	34	3960	44	40	29548	40	30628	34
762	48	39888	88	40	30	36	8416	1262	4096	1598	12372
52058	8050	2524	60	450	168	32	340	22410	534	1046	512
106	2080	172	170	38	38	30	444	7478	2458	10024	15470
30	46	16428	25792	4240	45094	2092	630	334	17130	388	2730
2498	732	358	5044	3236	202	2124	292	4806	13736	13012	12386
290	30	3986	886	4584	240	110	940	1286	162	6768	2204
456	816	438	294	260	44	524	40	34	22	38	20

Plate Repeat End time Start temp. End temp. BarCode  
6 1 ##### 22 22.1 N/A

0.5 CPS (CPS)

32

40	13240	38	7756	38	3368	24	40	36836	48	30392	36
914	34	44702	64	50	20	24	8048	996	3826	1714	11588
50234	9110	1574	46	536	270	20	382	21124	424	804	682
200	1888	240	108	28	46	30	288	9302	2154	14254	16620
46	28	17532	29872	5846	51810	2346	938	286	22146	612	3346
3048	862	206	5668	3686	166	2092	214	4384	15762	14404	12586
390	64	3518	926	4362	318	176	1032	924	156	6956	2938
672	810	314	242	252	26	378	30	20	34	50	38

R5 No Drug	X4										X4											
	No Drug					Drug					No Drug					Drug						
34 14,140 46 21,656	30 3,988 30 49 42,206	45 44,589 32 26,471	55 6,849 31 1,217	7,336 70 1,159	97 29,118	72 19,715	3,948 20 10,836	5,204 20 7,130	50	5,330 33 45	9,695 31	68 226 32 33	974 32 33	45 18 43	52 34	3,310 668 22 61	338 45 18 43	52 34	3,310 668 22 61	338 45 18 43	52 34	
4,5582 14,149 67,389	611 46 284	100 11,299 1,273	7,375 4,397	16,115 19,102	226 226	72 19,715	3,948 20 10,836	5,204 20 7,130	50	5,330 33 45	9,695 31	68 226 32 33	974 32 33	45 18 43	52 34	3,310 668 22 61	338 45 18 43	52 34	3,310 668 22 61	338 45 18 43	52 34	
67,828 14,982 3,788	50 688 231 53	177 24,739	612 2,987	416 68	12,020 4,384	37 242	35 61	338 45 18 43	52 34	3,310 668 22 61	338 45 18 43	52 34	3,310 668 22 61	338 45 18 43	52 34	3,310 668 22 61	338 45 18 43	52 34	3,310 668 22 61	338 45 18 43	52 34	
100 3,774 450	148 32 60 45	714 11,029	2,908 13,987	24,377 39	2,310 668	24 22	29 23,846	20,393 39 126	31	2,310 668 24 22	29 23,846	20,393 39 126	31	2,310 668 24 22	29 23,846	20,393 39 126	31	2,310 668 24 22	29 23,846	20,393 39 126	31	
35 83 18,099	39,257 5,413	97,733 5,249	19,175 513	3,264 107	4,209 8,451	38 40	51 77	34 41	38	4,209 8,451	38 40	51 77	34 41	4,209 8,451	38 40	51 77	34 41	4,209 8,451	38 40	51 77	34 41	
3,769 786 340	5,189 4,318	4,318 3,853	411 7,857	25,437 17,443	16,707 40 48	40 48	28 9,102	105 5,577	38 43	16,707 40 48	28 9,102	105 5,577	38 43	16,707 40 48	28 9,102	105 5,577	38 43	16,707 40 48	28 9,102	105 5,577	38 43	
391 98 4,449	4,357 6,090	1,886 3,890	263 3,890	2,089 4,475	8,475 4,107	26 38	33 3,815	3,635 9,577	38 43	8,475 4,107	26 38	33 3,815	3,635 9,577	38 43	8,475 4,107	26 38	33 3,815	3,635 9,577	38 43	8,475 4,107	26 38	
510 979 491	300 297	297 39 443	34 22 45	43 46	33 33	59 33	33 3,815	3,615 61,594	13,452 128,238	62 33	33 3,815	3,615 61,594	13,452 128,238	62 33	33 3,815	3,615 61,594	13,452 128,238	62 33	33 3,815	3,615 61,594	13,452 128,238	62 33
<b>L83</b>																						
30 54 29	27 32	34 27	37 90	35 60	40 40	19,256 9,956	32 153	4,696 14,985	25,542 14,985	5,468 2,953	28 2,918	3,058 3,397	33 3,397	934 33	32 33	761 6,455	46 3,874	22,279 38	33 3,874	7,261 33	33 3,874	7,261 33
28 40 99	30 21	38 32	32 22	40 42	68 68	10,839 10,839	38 43	10,839 10,839	3,397 3,397	60 60	221 221	43 43	828 65	33 65	33 36	54 54	33 36	54 54	33 36	54 54	33 36	
129 111 59	38 32	47 32	28 32	31 48	22 28	3,636 3,636	35 463	3,636 3,636	28 28	65 65	207 207	35 35	23 23	32 32	33 33	33 33	32 32	33 33	32 32	33 33	32 32	
29 38 33	30 32	32 31	31 18	31 73	59 59	3,502 3,502	34 304	3,502 3,502	34 34	33 33	9,150 9,150	34 34	8,290 8,290	33 33	33 33	33 33	33 33	33 33	33 33	33 33	33 33	
31 115 13,896	85 35 30	43 30	38 37	96 96	35 35	104 104	24 24	104 104	24 24	38 38	28 28	28 28	27 27	87 87	34 34	47 47	39 39	43 43	39 39	43 43	39 39	
29 39 31	34 23	28 39	23 39	42 42	35 35	40 40	35 35	40 40	35 35	45 45	40 40	7,932 7,932	77 77	2,092 2,092	58 58	6,140 6,140	1,224 1,224	50 50	44 44	30 30	44 44	
50 31 43	34 23	38 33	33 33	44 33	30 30	28 28	33 33	30 30	28 28	35 35	40 40	7,932 7,932	77 77	2,092 2,092	58 58	6,140 6,140	1,224 1,224	50 50	44 44	30 30	44 44	
42 55 49	29 32	32 25	33 33	35 33	36 36	20 27	27 27	20 27	27 27	42 42	55 55	3,661 3,661	2,946 2,946	56,739 56,739	12,051 12,051	85,018 85,018	33 33	39 39	37 37	25 25	39 39	37 37
<b>AMD</b>																						
39 12,186 32	7,226 45	3,664 25	40 33,192	44 3,961	1,636 1,636	11,980 925	34 597	11,980 925	34 39	3,384 3,384	598 49	33 49	3,384 598	34 49	34 34	35 35	42 42	37 37	40 40	33 33	37 37	
838 41 42,295	76 53	493 42	26 21,767	479 479	12,139 16,045	16,045 2,306	28 272	16,045 2,306	28 272	72 72	30 30	26 26	36 36	29 29	34 34	42 42	37 37	40 40	33 33	37 37	40 40	
51,146 8,580 2,049	53 30	139 33	361 2,767	784 784	19,638 500	5,038 3,495	34 8,711	19,638 500	34 8,711	35 35	43 43	33 33	44 44	36 36	45 45	42 42	37 37	40 40	33 33	42 42	37 37	
153 1,984 2,06	139 42	48,452 2,219	2,219 2,219	253 4,595	14,749 14,749	12,486 13,708	41 41	12,486 13,708	41 41	22 22	40 40	39 39	27 27	33 33	45 45	160 160	36 36	47 47	32 32	44 44	32 32	
38 37 16,980	27,832 5,043	48,452 2,219	184 2,108	986 1,105	1,159 1,159	6,862 2,571	35 35	6,862 2,571	35 35	27 27	28 28	44 44	29 29	36 36	51 51	55 55	45 45	51 51	45 45	51 51	45 45	
2,773 757 282	5,356 3,461	3,461 2,108	279 143	906 4,473	2,752 3,752	4,473 2,752	35 35	4,473 2,752	35 35	27 27	28 28	44 44	29 29	36 36	51 51	55 55	45 45	51 51	45 45	51 51	45 45	
340 47 3,752	906 4,473	4,473 2,752	268 266	376 356	356 356	451 451	35 35	451 451	35 35	27 27	28 28	44 44	29 29	36 36	51 51	55 55	45 45	51 51	45 45	51 51	45 45	
564 813 376	268 356	356 356	356 356	356 356	356 356	356 356	35 35	356 356	35 35	27 27	28 28	44 44	29 29	36 36	51 51	55 55	45 45	51 51	45 45	51 51	45 45	

Table 6

*T20 Resistance Mutations*

<u>SDM</u>	<u>virus</u>	<u>T20 sens.</u> <sup>a</sup>	<u>T20</u>	<u>FC<sup>b</sup></u>
HXB2	G T V	S	1.0	
NL4-3	G T V	S	5.2	
NL4-3	D T V	S	12.8	
NL4-3	G T M	S	33.0	
NL4-3	S T V	S	74.2	
NL4-3	D T M	R	113.0	
NL4-3	S T M	R	227.4	
NL4-3	D T V	R	>281.8	
JRCSF	G T V			2.1
JRCSF	D T V			104.0

<sup>a</sup> Rimsky et al., J. virol. 72(2):986-993<sup>b</sup> Fold change in IC50 (vs. HXB2) using Phenosense HIV Entry Assay

### **T20 Resistance Mutations**

<u>SDM Virus</u>	<u>T20 Sens.<sup>a</sup></u>	<u>T20 FC<sup>b</sup></u>
HXB2 G I V		1.0
NL4-3 G I V	S	5.2
NL4-3 D I V	S	12.8
NL4-3 G I M	S	33.0
NL4-3 S I V	S	74.2
NL4-3 D I M	R	113.0
NL4-3 S I M	R	227.4
NL4-3 D T V	R	>281.8
JRCSF G I V		2.1
JRCSF D I V		104.0

<sup>a</sup> Rimsky et al., J. Virol. 72(2):986-993

<sup>b</sup> Fold change in IC50 (vs. HXB2) using PhenoSense HIV Entry Assay

**Table 6**

#### Entry Inhibitor Susceptibility: T-20 Fusion Inhibitor

Drug resistance mutations were introduced into well-characterized X4 tropic (NL4-3) and R5 tropic (JRCSF) viruses. T20 susceptibility was measured using the virus entry assay. The fold change (FC) in T-20 susceptibility for each virus was determined by dividing the IC50 of the test virus by the IC50 of the HXB2 strain of HIV-1. T-20 sensitivity of similar mutant viruses has been reported in the scientific literature (Rimsky et al.,).

Table 7

# Identifying Entry Inhibitor Resistance Mutations

Virus	HXB2	AA Sequence <sup>a</sup>											
		X=G/S	X=G/M	X=G/D	X=G/S	X=G/M	X=G/D	X=G/S	X=G/M	X=G/D	X=G/S	X=G/M	X=G/D
1		Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
2		L	S	G	V	Q	Q	Q	Q	Q	Q	Q	Q
3		L	S	G	V	X	V	V	X	V	V	V	V
4		L	S	G	I	I	I	I	I	I	I	I	I
5		L	S	X	I	I	I	I	I	I	I	I	I
6		L	S	G	I	I	I	I	I	I	I	I	I
7		L	S	G	X	I	I	I	I	I	I	I	I
8		L	S	G	S	G	I	I	I	I	I	I	I
9		L	S	G	S	G	I	I	I	I	I	I	I
10		L	S	G	S	G	I	I	I	I	I	I	I
11		L	S	G	S	G	I	I	I	I	I	I	I
12		L	S	G	S	G	I	I	I	I	I	I	I
13		L	S	G	S	G	I	I	I	I	I	I	I

<sup>a</sup> gp41 amino acid sequence positions 32 to 41

### Identifying Entry Inhibitor Resistance Mutations

<u>Virus</u>	<u>AA Sequence<sup>a</sup></u>	
HXB2	Q L L S G I V Q Q Q	
1	Q L L S G I V Q Q Q	
2	Q L L S S I M Q Q Q	
3	Q L L S X I X Q Q Q	X=G/S X=V/M
4	Q L L S G I V Q Q Q	
5	Q L L S G I V Q Q Q	
6	Q L L S X I X Q Q Q	X=G/S X=V/M
7	Q L L S G I V Q Q Q	
8	Q L L S G I V Q Q Q	
9	Q L L S G I V Q Q Q	
10	Q L L S D I V Q Q Q	
12	Q L L S X I V Q Q Q	X=G/D
13	Q L L S G I V Q Q Q	

<sup>a</sup> gp41 amino acid sequence positions 32 to 41

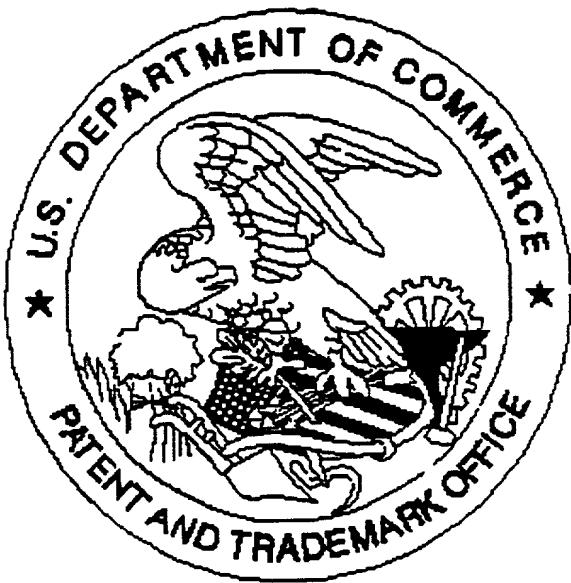
**Table 7**

#### Identifying Envelope Protein Mutations.

Viruses with reduced (or increased) susceptibility to an entry inhibitor are identified using the virus entry assay. Mutations that may confer reduced (or increased) susceptibility to the entry inhibitor are identified by sequencing the envelope genes of the sensitive and resistant viruses. The deduced amino acid sequences of the sensitive and resistant viruses are compared to identify candidate drug resistance mutations. The ability of a specific mutation to confer altered drug susceptibility is confirmed or disproved by introducing the mutation into a drug sensitive virus and measuring the susceptibility of the mutant virus in the virus entry assay. In the example represented here, a short stretch of amino acid sequences within the first heptad repeat (HR-1) of the HIV-1 gp41 transmembrane envelope protein is aligned for viruses exhibiting different T-20 susceptibilities. Highlighted amino acids represent mutations known to confer reduced susceptibility to T-20.

Similar analyses can be used to identify envelope amino acid sequences that (a) alter/influence susceptibility to CCR5 or CXCR4 inhibitors, (b) specify X4, R5 and dual tropism, and (c) elicit neutralizing antibodies.

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for scanning. (Document title)

They are 8 drawings not 13 pages

*Scanned copy is best available.* Drawings and Miscellaneous